Functional variants of 17q12-21 are associated with allergic asthma but not allergic rhinitis

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Background: Allergic rhinitis (AR) and asthma are common allergic conditions with a shared genetic component to their cause. The 17q12-21 locus includes several genes that have been linked to asthma susceptibility, but the role of this locus in AR is unclear. Asthma and AR in adults of Chinese ethnicity in Singapore are predominately caused by sensitization against house dust mites with a nearly complete penetrance of the allergen, which presents a unique opportunity for accurately identifying genetic associations with allergic diseases.

Objective: We sought to define the functional role of 17q12-21 in patients with AR and allergic asthma.

Methods: We asked whether single nucleotide polymorphisms (SNPs) in the 17q12-21 locus were associated with AR or asthma in a cohort of 3460 ethnic Chinese subjects residing in Singapore (1435 in the discovery phase and 2025 in the validation phase). Full-blood mRNA gene expression data, plasma IgE levels, and immune cell frequencies in peripheral blood were tested against the tag SNP genotypes. Luciferase assays were used to measure the effect of putative promoter SNPs on expression of the asthma-associated orosomucoid-like 3 gene *(ORMDL3)*.

Results: Within 17q12-21, only the tag SNP rs8076131 was significantly associated with asthma ($P = 8.53 \times 10^{-10}$; odds ratio, 0.6715), and AR status was independent of SNPs in this region. C-A alleles at rs8076131 resulted in significantly increased *ORMDL3* expression in HEK293 cells *in vitro* relative to T-G alleles. Moreover, subjects with the risk genotype AA exhibited significantly higher total IgE levels and higher blood eosinophil counts than those with the lower-risk genotypes.

Conclusion: The 17q12-21 locus has a strong genetic association with allergic asthma but not with AR. The polymorphic effect of this locus is attributed to the linkage set tagged by rs8076131, which affects the expression of *ORMDL3*, protein phosphatase 1, regulatory inhibitor subunit 1B (*PPP1R1B*), zona pellucida binding protein 2 (*ZPBP2*), and gasdermin B *(GSDMB)* and is correlated with high IgE levels and eosinophil counts in subjects bearing the risk genotype. (J Allergy Clin Immunol 2015;■■■:■■■-■■■.)

Key words: *ORMDL3, 17q21, allergic asthma, allergic rhinitis, Singapore Chinese, IgE, eosinophil counts*

In recent decades, the prevalence of allergic disease has increased steeply across the globe.[1-4] Environmental changes are an important contributor to this increase,[5] but the high heritability of allergic conditions also indicates a strong genetic component to their cause.[6,7] The common co-occurrence of allergic rhinitis (AR) and asthma in the same subject further suggests the existence of shared susceptibility loci[8]; one candidate is chromosome region 17q12-21, a 300-kb stretch of the genome encompassing 12 genes, including the asthma genes of interest orosomucoid-like 3 gene *(ORMDL3)*, gasdermin B *(GSDMB)*, IKAROS family zinc finger 3 (Aiolos *[IKZF3]*), and zona pellucida binding protein 2 (*ZPBP2*; Fig 1). These genes have wide-ranging functions and have been implicated in various aspects of the pathogenesis of asthma and allergy: Orm-like proteins (ORMDL1, ORMDL3, and ORMDL4) can mediate sphingolipid homeostasis, which is thought to be dysregulated in asthmatic patients[9,10]; *GSDMB* is a member of the gasdermin *(Gsdm)* family of genes, which are expressed in epithelia and have been linked with stem cell proliferation[11,12]; *IKZF3* belongs to the Ikaros family of zinc-finger proteins, which are transcription factors regulating lymphocyte development[13]; and *ZPBP2* encodes a secreted-type glycoprotein expressed in germ cell tumors, the testis, and brain medulla.[14] The 17q21 locus containing these genes was first linked to pediatric asthma susceptibility by Moffatt et al[15] and has since been confirmed as an asthma-associated locus in various other populations.[16-18] Although some reports have suggested that 17q12-21 is also a susceptibility locus for AR,[19-21] others have been unable to confirm the association.[22-24] This study investigates the role of 17q21 in asthma and AR for the Chinese population.

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Abbreviations used
AR: Allergic rhinitis
eQTL: Expression quantitative trait loci
GSDMB: Gasdermin B
HDM: House dust mite
IKZF3: IKAROS family zinc finger 3 (Aiolos)
OR: Odds ratio
ORMDL3: Orosomucoid-like 3 gene
SNP: Single nucleotide polymorphism
SPT: Skin prick test
ZPBP2: Zona pellucida binding protein 2

The majority of cohorts used in genome-wide association studies of allergic conditions unavoidably have high rates of false-negative results because of the diversity of allergens and the heterogeneity of individual responses. This effect can obscure true associations and make accurate interpretation of the results challenging. We previously showed that in the tropical urban environment of Singapore, the typically complex allergen sensitization profile is reduced to monosensitization by house dust mite (HDM).[25] Skin prick tests (SPTs) with the 12 most common allergens revealed that 98% of subjects with positive SPT responses react to HDM, with two thirds of the cohort reacting exclusively to this allergen. Moreover, because of a strong environmental penetrance, nearly 80% of the Chinese ethnicity population responds to HDM sensitization, which is associated with a prevalence of AR and asthma of about 40% and 15%, respectively.[25] This clearly defined population and environment thus represents a unique framework within which to accurately define the role of genetic polymorphisms in patients with allergic disease. Here we conducted a cohort-based correlation study on approximately 3500 adults of Chinese ethnicity residing in Singapore to reveal the true role of 17q12-21 in susceptibility to AR and asthma.

METHODS

Samples

The samples used in this study were collected with approval of the appropriate institutional review boards in Singapore. Recruitment was performed in compliance with the Helsinki Declaration. In addition, parental/guardian consent was obtained for all participants less than 21 years of age.[22] We used a 2-stage design of discovery and validation, both of which were part of an ongoing epidemiologic study on allergies. All samples were collected by using the same study protocol and in the same ethnic Chinese population in Singapore. Demographics of the samples are provided in Table I. Volunteers were of Chinese ethnicity and resident in Singapore, and DNA was extracted from mouthwash or whole blood. The ethnicity of the donors was self-reported; however, their Chinese ethnicity was confirmed previously by using principal component analysis because it was similar to the Han Chinese (CHB) ethnicity from the HapMap project.[22,26] All participants completed an International Study of Asthma and Allergies in Childhood/Allergic Rhinitis and its Impact on Asthma–based questionnaire for allergy and underwent SPTs to determine their sensitization to a panel of allergens commonly found in Singapore, including the HDM allergens from *Dermatophagoides pteronyssinus* and *Blomia tropicalis*, as well as *Elaeis guineensis* and *Curvularia lunata*. An SPT response was considered positive when a wheal of at least 3 mm in diameter was observed 15 minutes after the skin prick. Histamine and saline were used as positive and negative controls, respectively. The AR diagnostic criteria used were 2 or more self-reported symptoms of AR (nasal congestion, rhinorrhea, nasal itching, and sneezing) persisting for 4 or more days a week accompanied by a positive SPT response for HDM.[22,27] Allergic asthma was defined based on a self-reported doctor's diagnosis of asthma and a positive SPT response for HDM.[28,29] ImmunoCAP was used to measure total IgE levels in plasma samples from volunteers.[25] Gene expression within the 17q12-21 locus was evaluated by using whole-blood expression quantitative trait loci (eQTL) estimated from a cohort in Singapore. Data were available for a subset of the genotype samples.[25,28] The various cohorts used in the study are described in Fig E1 in this article's Online Repository at www.jacionline.org.

Tag single nucleotide polymorphism selection

A total of 10 tag single nucleotide polymorphisms (SNPs) were selected to tag the chromosome 17q12-21 locus, with a minor allele frequency of 5% using the Hap Map Chinese Han in Beijing (CHB) population. A linkage disequilibrium threshold of an r^2 value of 0.8 was used to tag SNPs for association analysis by using the tagger algorithm.[30]

Genotyping

The amount of DNA isolated from patients' samples was measured in triplicate on a NanoDrop (ND 1000; Thermo Scientific, Wilmington, Del) before use. Genotyping for the discovery phase was performed on purified DNA samples by using the Illumina Bead Xpress Assay (Illumina, San Diego, Calif), according to the manufacturer's recommendations, at the University of Utah Genomics Core Facility (Salt Lake City, Utah). In the validation phase samples were genotyped with the Sequenom platform with MassARRAY and iPLEX technology (Sequenom, San Diego, Calif). Oligonucleotides were designed according to Sequenom guidelines by using MassARRAY Assay Design software. Amplicons containing SNPs of interest underwent multiplex PCR, followed by primer extension reactions. Data were analyzed with Sequenom TYPER software. Extent of clustering of genotype calls was used to select samples for inclusion in the statistical analysis.

Luciferase assay in HEK293T cells

Human embryonic kidney cells (HEK293T) were purchased from the American Type Culture Collection (ATCC, Manassas, Va) and grown in RPMI-1640 medium (Sigma-Aldrich, Singapore) with 2 g/L sodium bicarbonate, 2 mmol/L L-glutamine, and 10% FBS. Cells were grown at 37°C in a 5% CO_2 atmosphere in air in a humidified incubator.

The haplotype effect of selected SNPs on *ORMDL3* gene expression was measured by using the luciferase assay. The region spanning +1906 bp to +3392 bp of *ORMDL3* was cloned into a promoter-less pGL4.10 vector containing a firefly luciferase reporter gene (Promega, Singapore). Plasmid constructs were transiently transfected into HEK293T cells by using Lipofectamine 2000 according to the manufacturer's protocol (Invitrogen, Singapore). Cotransfection with a renilla luciferase construct with HSV-TK promoter (plasmid pGL4.74) was used to control for variations in transfection efficiency; accordingly, the firefly luciferase reading was normalized against that of renilla luciferase. All experiments were performed in triplicate, with the luciferase reading measured at 24 or 48 hours after transfection. An independent samples *t* test was used to calculate the *P* value of the average difference in gene expression level induced by each allele of the SNP.

Whole-blood eQTL data: Singapore Chinese

We analyzed whole-blood gene expression data from 71 Chinese ethnicity volunteers in the context of their whole-genome SNP profile (data from another ongoing study). mRNA was extracted from whole blood collected into tempus RNA tubes (Life Technologies, Carlsbad, Calif), and transcript abundance was measured by using the Illumina HumanHT-12-v4 Expression Bead Chip (Illumina, San Diego, Calif). The Illumina Human Omni5Quad chip was used to determine the genome-wide SNP profile. Only Illumina probes free of any SNPs were used to determine the expression level of the genes to avoid allele-specific artifacts. Probes used for analysis included the following: ILMN_1657095 (StAR-related lipid transfer [START] domain containing 3 [*STARD3*]), ILMN_1662174 (ORMDL sphingolipid biosynthesis regulator 3